



**NOAA
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9.2 What is epigenetics and why could it be relevant to recovery and management of protected resources?

Penny Swanson, Krista Nichols

Northwest Fisheries Science Center

Mackenzie Gavery

JISAO, University of Washington

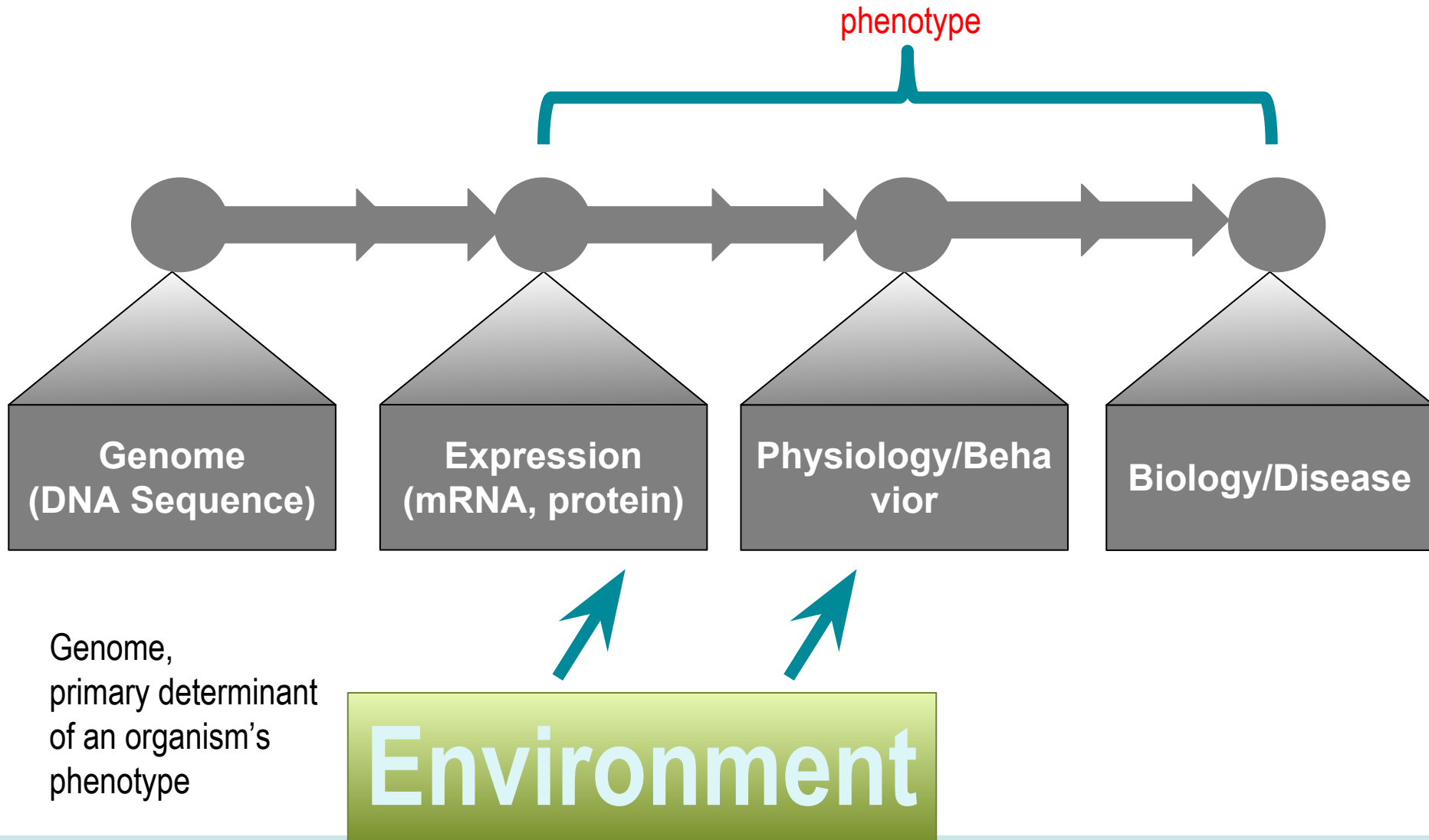
Funded by Bonneville Power Administration
and NOAA Fisheries

May 6, 2015

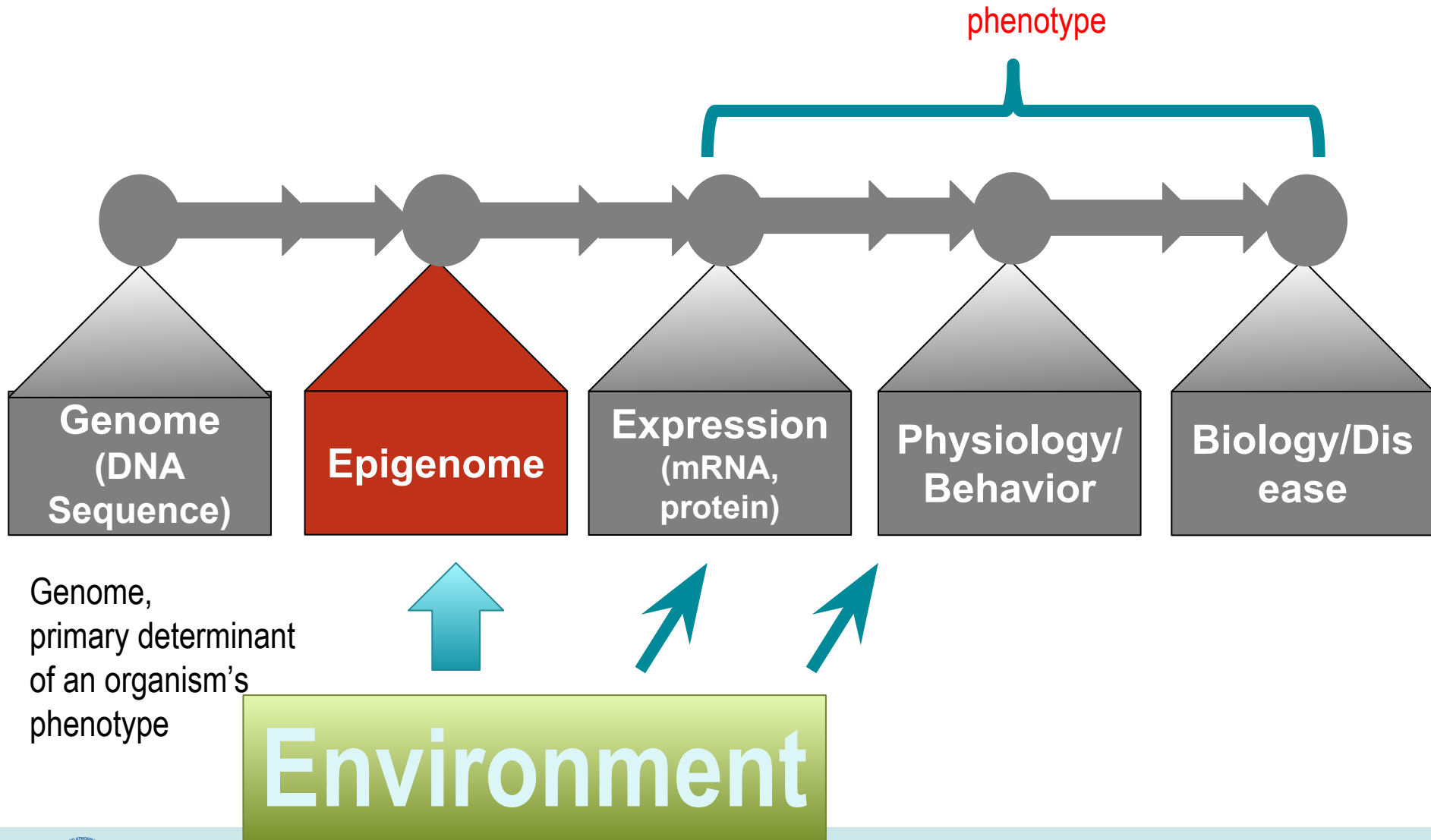
Problem

- Hatchery programs may reduce the “fitness” of salmon and steelhead spawning in the nature
- Plausible mechanisms:
 - Hatchery-induced selection that causes rapid genetic change
 - Environmentally-induced changes in juveniles that alter:
 - Adult phenotype and reduce breeding success
 - Non-genetic factors in germ cells that affect offspring survival
 - Both

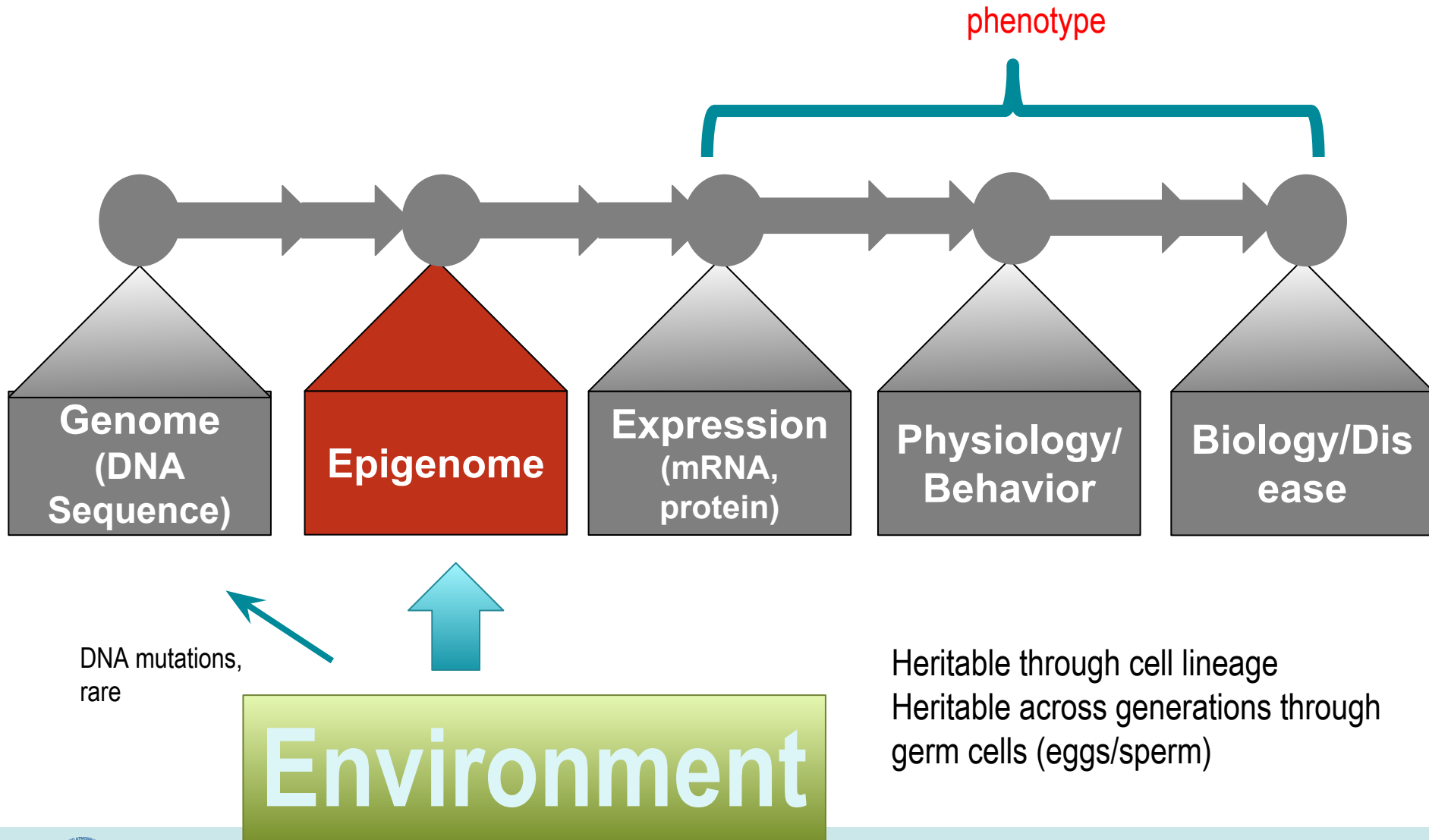
Genetic x Environment ➔ Phenotype



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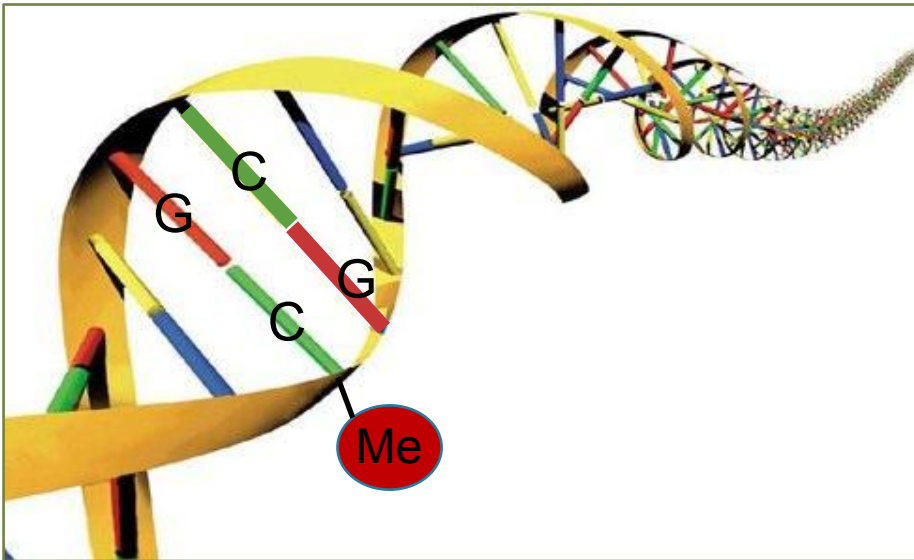


Heritable changes that affect phenotype



DNA Methylation

- Most well understood epigenetic mechanism is **DNA methylation**
 - Methylation of CpG occurs in most plants and animals
 - Typically* associated with gene silencing if in promotor region of gene
 - Methylation in gene bodies associated with enhanced transcription, formation of splice variants



Examples of change in DNA methylation associated with phenotypic change

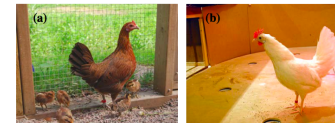
- Temperature- sex change
- Aging- twins
- Domestication-stress response
- Behavior- maternal care, anxiety
- Nutrition/Toxins-coat color, obesity



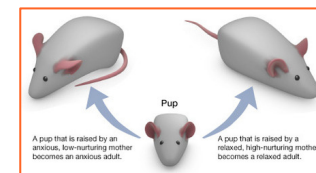
PLoS Genet 7: e1002447



PNAS (2005)102:10604-10609



BMC Genomics (2012) 13: 59



<http://learn.genetics.utah.edu/content/epigenetics/rats/>

Nature Neuroscience (2004) 7:847-854



Environ. Health Perspect (2006)114:567-572

Implications for Evolution

- Genome changes slowly, through random mutation and natural selection on the phenotype. Takes many generations for genetic trait to be common in a population
- Epigenome changes rapidly in response to signals from the environment and can happen in many individuals at once. Some MAY be passed on to future generations. Through epigenetic inheritance, experiences of parents may pass to future generations.
- Epigenome is flexible, allows organism to continually adjust gene expression according to environment without changing DNA code

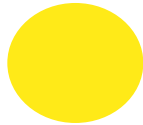
Why could epigenetics be relevant to understanding loss of fitness of hatchery fish, or rapid adaptation and evolution?

Hatchery rearing environment differs from nature and is manipulated to meet production goals

- Embryo incubation temperature- alters seasonal timing of emergence & ponding of fry
- Energy content of diet higher than natural diet
- Seasonal pattern of juvenile growth different than in nature
- Rearing density higher (can affect stress)
- Lack of predators

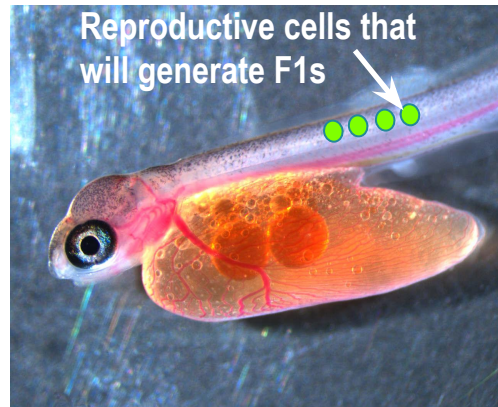
Potential factors that could induce heritable changes in the epigenome

photoperiod



temperature

Olfactory cues



Hormones in the yolk

*Water chemistry
(pH, organics,
toxins, O₂)*

*Nutrients
(yolk and exogenous
food)*

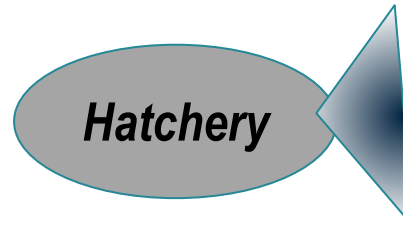
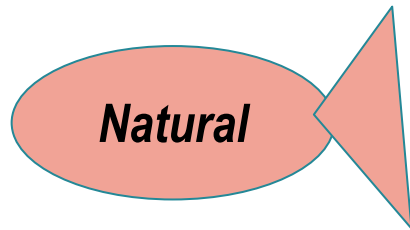
Questions

Are there discernable epigenetic and/or genetic differences between hatchery and natural origin steelhead that could be passed to subsequent generations?

If so, does it differ between hatchery rearing protocols (S1 vs S2 smolt)?

Experimental Design:

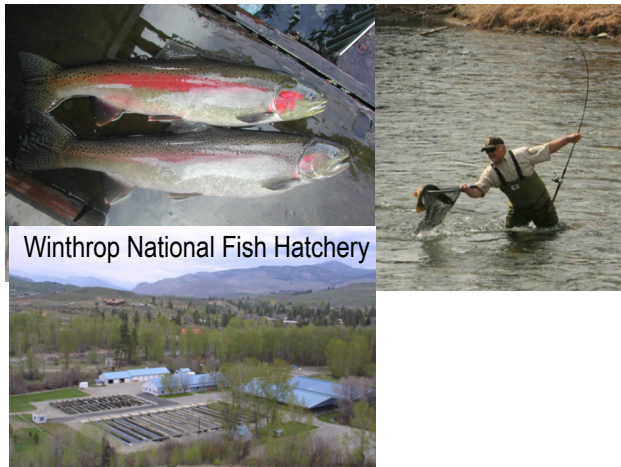
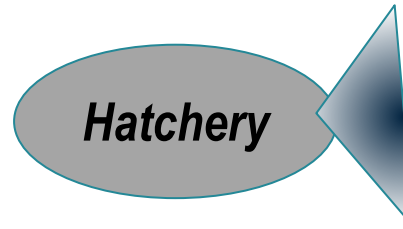
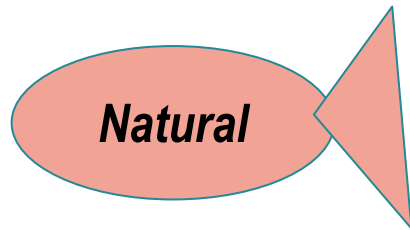
Compare genetic and epigenetic variation in natural and hatchery origin Methow River steelhead



- Returning Methow River steelhead adults collected by angling
- Collect fin clip, blood, gametes
- **Genetic analysis:** Genome wide SNP analysis, 15,000 SNPs, RAD sequencing
- **Epigenetic analysis:** Genome wide DNA methylation analysis in sperm, RRBS

Experimental Design:

Compare genetic and epigenetic variation in natural and hatchery origin Methow River steelhead



- Returning Methow River steelhead adults collected by angling
- Collect fin clip, blood, gametes

No discrimination between natural & hatchery origin fish

- **Epigenetic analysis:** Genome wide DNA methylation analysis in sperm, RRBS

Preliminary Results: Epigenetics

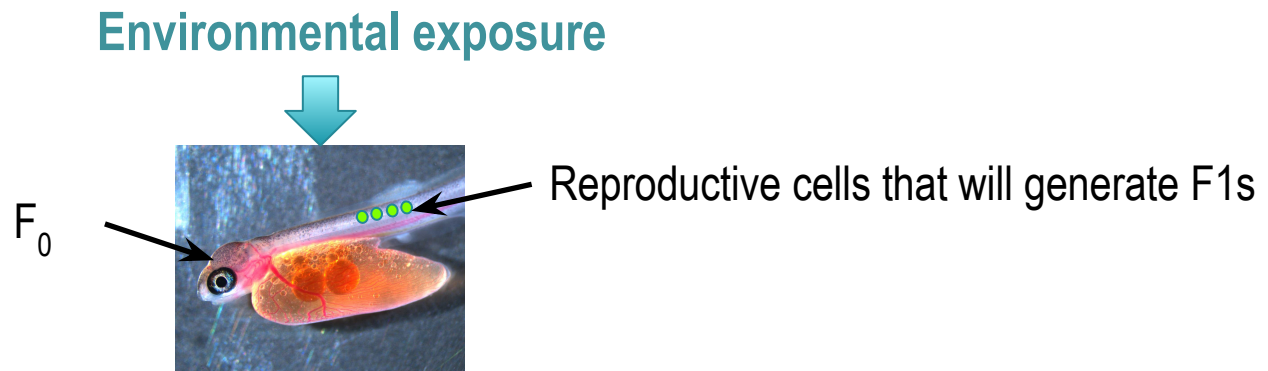
- 74 differentially methylated CpG Regions (DMRs) in sperm DNA
 - 45 hypermethylated in hatchery fish
 - 29 hypomethylated in hatchery fish
- Almost half of the DMRs are located within 5kb of genes (potential gene regulatory regions), one third within gene bodies
- Differences could be due to early rearing environment, but cannot rule out age.
- Currently analyzing additional samples- comparing two hatchery rearing methods (yearling vs 2-year old smolt) allows us to test for age and rearing environment effects

What about transgenerational effects?

Establishing epigenetic differences in sperm DNA from natural and hatchery origin fish is compelling, but does not prove these are heritable.

Challenges of Proving Transgenerational Epigenetic Inheritance

- Must rule out possibility of genetic change
- Must show that the epigenetic effect can pass through several generations to rule out possibility of direct exposure



New Study- Transgenerational Epigenetic Inheritance

Hypothesis- Early Rearing Environment Alters Epigenetic Programming of Steelhead Germ Line

20 families split across two rearing environments

Hatchery



Artificial Stream



Phenotype of Steelhead from Hatchery vs Artificial Stream Environment: 8 months of rearing

Hatchery



Artificial Stream



Bar = 100 mm

Next steps: 1-2 more years of rearing needed to obtain maturing males for sperm DNA methylation analysis. Test for rearing environment effects. Compare paternal and offspring methylation patterns.

Summary

- Assembled a strong interdisciplinary team to investigate role of epigenetics in phenotypic plasticity, adaptation and evolution in *O. mykiss*
 - *Expertise in molecular and quantitative genetics, functional genomics, fish physiology, behavioral ecology, and bioinformatics*
 - *Facilities for lab studies, hatchery rearing, computer cluster for bioinformatics*
 - Partnerships with USFWS, UW, WSU, and Institut National de la Recherche Agronomique (INRA)
- Established methodology for analyzing DNA methylation and bioinformatic pipelines to analyze data
- Found significant differences in methylation of sperm DNA due to hatchery rearing or age. Suggest potential for heritable epigenetic effects of early rearing environment.
- Studies initiated to test for transgenerational epigenetic inheritance

What are the challenges?

- Bioinformatics challenging because of genome duplications in salmonines
- Data analyses in other Pacific salmon species without genome sequence difficult
- Linking specific differences in epigenetic programming to functional differences, phenotypes
- Costs of the analysis
- Long-term funding for transgenerational studies in species with 2-4 year generation times

Where could this research go?

- Reforming hatchery rearing practices (diet, feeding regimes, early rearing protocols) to reduce impacts
- Development of epigenetic signatures of wild and hatchery fish of same stock that cannot be discriminated with genetic analysis
- Understanding scope of adaptation to extreme changes in environment
- Numerous applications to habitat restoration and toxicological research
- If epigenetic changes occur, can they readjust in several generations in the wild?
Epigenome is more dynamic than genome.



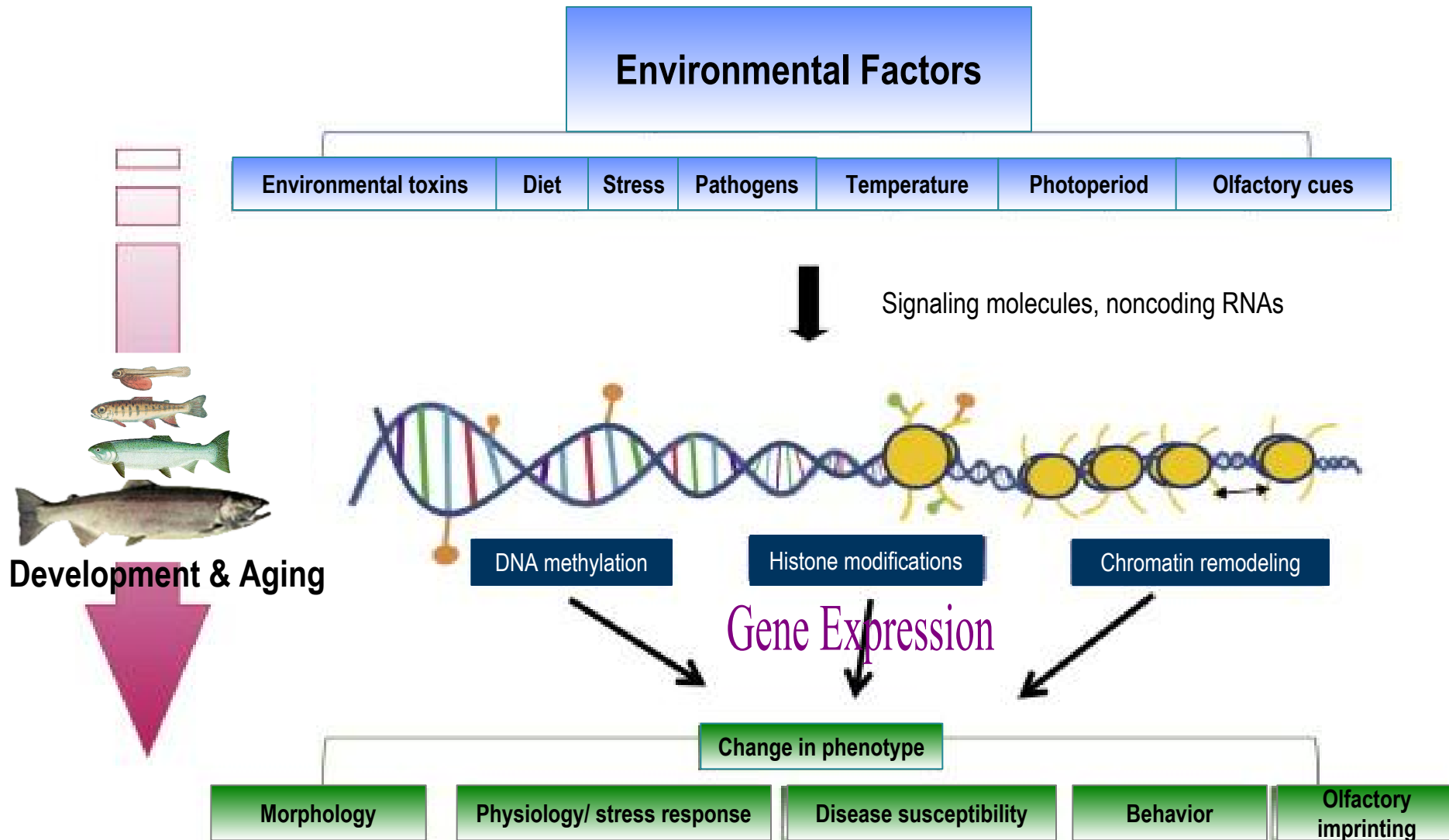
Acknowledgements

- Funding from Bonneville Power Administration & NOAA Fisheries
- Mollie Middleton, University of Washington
- Barry Berejikian, Chris Tatara, Jeff Atkins, NOAA
- Chris Pasley, USFWS-WNFH
- Michael Humling, USFWS-WNFH
- Numerous volunteers that caught fish
- Mike Skinner, Washington State University

Extra slides

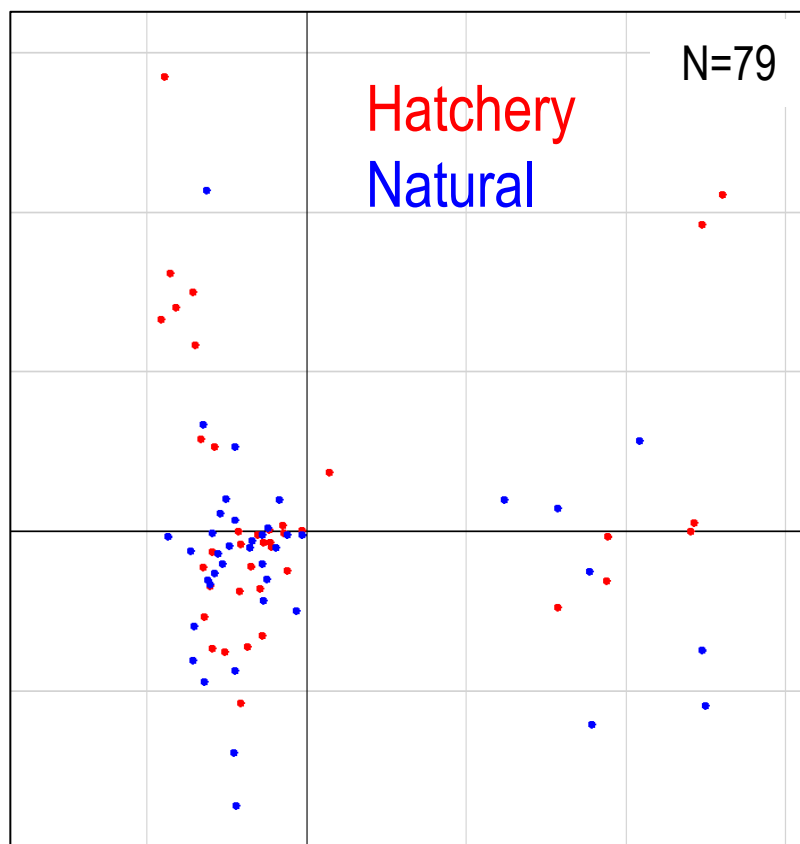


Epigenetic Mechanisms: Link Between Nature and Nurture



Results: Genetics

Genetic Analysis of Natural and Hatchery Origin Methow River Steelhead: Genome Wide Assessment of SNPs

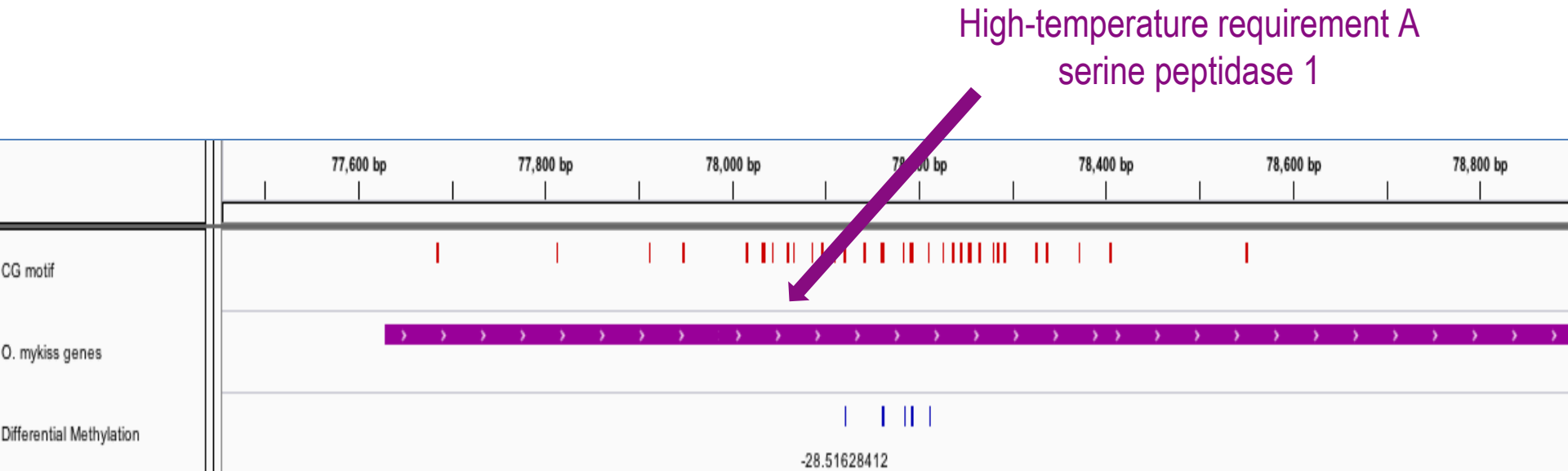


Preliminary results:

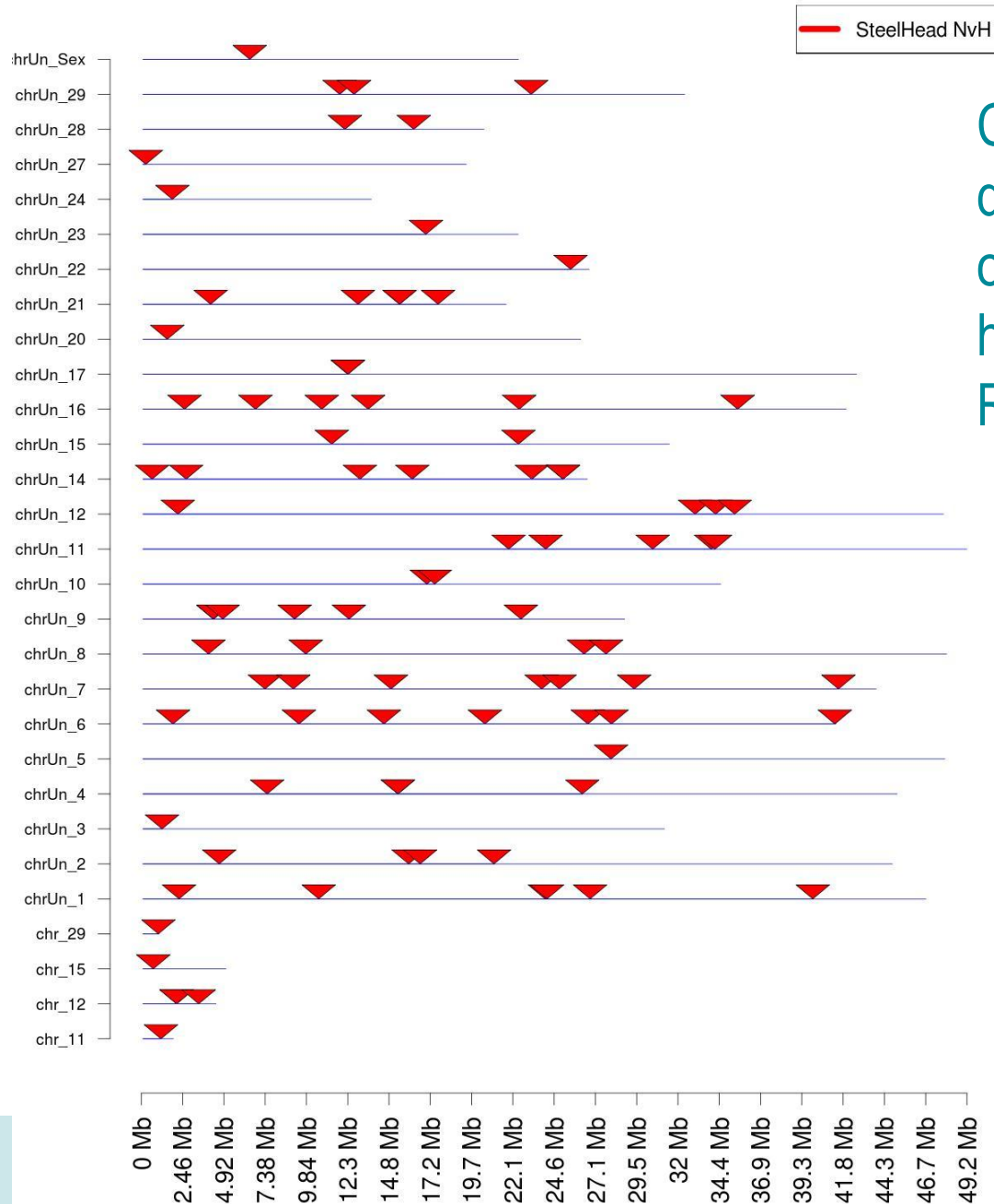
- 15,000 SNPs analyzed
- No obvious discrimination between hatchery and natural fish
- No association with brood year/age, rearing environment, or sex

Example of gene with DMR:

HTRA1, regulates insulin like growth factor (IGF) availability,
cleaves IGF binding proteins



SteelHead Natural vs Hatchery 1e-04 3+ sites



Data generated in collaboration with Prof. Mike Skinner, WSU